PATENT
Attorney Docket No.: 015280-169300
Client Ref. No.: E-174-93/0

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of:

Louis E. Henderson, *et al.*

Application No.: 09/431,607

Filed: November 1, 1999

For: METHOD FOR IDENTIFYING AND USING COMPOUNDS THAT INACTIVATE HIV-1 AND OTHER RETROVIRUSES BY ATTACKING HIGHLY CONSERVED ZINC FINGERS IN THE VIRAL NUCLEOCAPSID PROTEIN

Examiner: Shanon A. Foley

Art Unit: 1648

DECLARATION UNDER 37 CFR § 1.132

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

I, Louis E. Henderson, being duly warned that willful false statements and the like are punishable by fine or imprisonment or both, under 18 U.S.C. § 1001, and may jeopardize the validity of the patent application or any patent issuing thereon, state and declare as follows:

1. All statements herein made of my own knowledge are true and statements made on information or belief are believed to be true.

2. I am an employee of SAIC under contract to the National Cancer Institute at the National Institutes of Health in Frederick, Maryland, in the AIDS Vaccine Program. I have been in this position for 15 years.

3. A substantial part of my work focuses on viral zinc fingers as a target for antiviral chemotherapy. Accordingly, I am an expert in the field of the invention, including the biology of retroviruses and the inactivation of retroviruses using various chemical agents targeted against retroviral zinc fingers. My *Curriculum Vitae* is of record.

4. I have reviewed and analyzed the above-referenced patent application, and I am familiar with the contents therein. In addition, I have read the Office Action dated June 16, 2004, received in the present case.

5. It is my understanding that the Examiner has rejected claims 24-29 under 35 U.S.C. § 112, first paragraph, as allegedly containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the art that the Applicants, at the time the application was filed, had possession of the claimed invention. In particular, the Examiner alleges that the specific exclusion of 5,5-Dithiobis(2-Nitrobenzoic Acid) ("DTNB") from the claims constitutes new matter. However, for the reasons set forth below, the Examiner's concerns are overcome.

6. It is my understanding that claim 24 has been amended as follows: (1) to delete the proviso explicitly excluding DTNB; and (2) to recite a composition comprising an inactivated ***intact*** retrovirus, wherein the ***intact*** retrovirus is inactivated due to disruption of one or more CCHC zinc fingers in a nucleocapsid ("NC") protein by contact with one of the compounds recited in claim 24, such as a disulfide compound. For the reasons set forth herein, it is my understanding that any NC zinc finger-disrupting compound that has no effect on inactivating an ***intact*** retrovirus (*e.g.*, DTNB) falls outside the scope of claim 24 as amended.

7. I believe that the specification, *e.g.*, Example 4, describes a specific set of tests for readily determining whether a disulfide compound of interest is capable of inactivating an ***intact*** retrovirus by disrupting NC zinc fingers. As a result, any disulfide compound such as DTNB that has no effect on inactivating an ***intact*** retrovirus simply falls outside the scope of claim 24 as amended.

8. In the first test, a disulfide compound of interest is incubated with ***purified, recombinant*** NC protein. The ability of the disulfide compound to oxidize, *i.e.*, disrupt, the CCHC zinc fingers in ***purified, recombinant*** NC protein is determined by monitoring the formation of NC cross-links. The results shown in Table 2 under "Protein (HPLC)" indicate that a wide variety of the 43 disulfide compounds tested disrupt the CCHC zinc fingers in ***purified, recombinant*** NC protein. As such, this test provides a simple initial screen to identify candidate disulfide compounds with NC cross-linking activity. However, this test does not indicate whether disulfide compounds capable of cross-linking ***purified, recombinant*** NC protein will also be effective at inactivating ***intact*** retroviruses.

9a. In the second test, the disulfide compound is incubated with ***intact*** retroviruses. The ability of the disulfide compound to inactivate ***intact*** retroviruses, *i.e.,* by penetrating the viral envelope and disrupting NC protein structure, is determined, for example, by: (1) visualizing

the formation of NC multimers using Western blot analysis (*e.g.,* Figure 15A); (2) measuring the time required to cross-link half of the NC protein (*e.g.,* Table 2 under "X-link T-½ (min)"); and (3) measuring the concentration of the disulfide compound required to inactivate half of a standardized number of ***intact*** retroviruses in a tissue culture infectivity assay (*e.g.,* Figure 15B).

9b. For example, Figure 15A shows the NC cross-linking activity of 12 disulfide compounds on ***intact*** HIV-1 retroviruses. Both ***intact*** HIV-1 retroviruses alone ("HIV-1," lane 1) and those exposed to N-ethylmaleimide ("NEM," lane 2) were used as controls. Strikingly, the appearance of NC multimers and/or the disappearance of NC monomers, dimers, trimers, and tetramers was associated with exposure of ***intact*** HIV-1 retroviruses to each of the 9 disulfide compounds listed in lanes 6-14, *i.e.,* Formamidine Disulfide ("C4b," lane 6), 2,2 Dithiobis(benzonitrile) ("C1d," lane 7), Aldrithiol-2 ("D1b," lane 8), 2,2-Dithiobis(Pyridine N-Oxide) ("E1d," lane 9), Tetramethylthiuram Disulfide ("E1b," lane 10), Tetraethylthiuram Disulfide ("FDA," lane 11), Dicyclopentamethylenethiuram Disulfide ("E4b," lane 12), Tetraisopropylthiuram Disulfide ("C3d," lane 13), and Tetrabutylthiuram Disulfide ("C4d," lane 14). This result indicates that these disulfide compounds are highly effective at penetrating the viral envelope and disrupting NC protein structure in ***intact*** HIV-1 retroviruses. By contrast, NC protein structure in ***intact*** HIV-1 retroviruses exposed to 5,5'-Dithiobis(2-Nitrobenzoic Acid) ("DTNB," lane 5) was nearly indistinguishable from controls. Although DTNB has excellent NC cross-linking activity, it is membrane-impermeant. As such, DTNB cannot penetrate the viral envelope to reach and disrupt NC protein structure in ***intact*** HIV-1 retroviruses. NC protein structure in ***intact*** HIV-1 retroviruses exposed to 4-(Dimethylamino)phenyl Disulfide ("B2d," lane 3) or Benzoyl Disulfide ("A3c," lane 4) was also nearly indistinguishable from controls. However, unlike DTNB, B2d and A3c are membrane-permeant, but have poor NC cross-linking activity. As a result, in this test, the amount of NC multimers formed were below the limit of detection.

9c. The graph in Figure 15B[1] extends the findings of Figure 15A by showing the association between viral envelope penetration and NC cross-linking activity on the inactivation of ***intact*** HIV-1 retroviruses in a tissue culture infectivity assay. In particular, the highly effective ability of C4b, C1d, and D1b to penetrate the viral envelope and disrupt NC protein structure in ***intact*** HIV-1 retroviruses was associated with the ability of increasing concentrations of C4b (open diamond), C1d (filled square), and D1b (filled circle) to inactivate ***intact*** HIV-1 retroviruses. By contrast, the inability of DTNB to penetrate the viral envelope in ***intact*** HIV-1 retroviruses was associated with the inability of DTNB (open circle with dashed line) to inactivate ***intact*** HIV-1 retroviruses, even at the highest concentration tested. The poor ability of membrane-permeant B2d and A3c to disrupt NC protein structure in ***intact*** HIV-1 retroviruses was associated with the need for higher concentrations of B2d (filled diamond)

and A3c (open square) to inactivate ***intact*** HIV-1 retroviruses. As such, these results show that membrane-permeant disulfide compounds with either poor (*e.g.*, B2d, A3c) or excellent (*e.g.*, C4b, C1d, D1b) NC cross-linking activity were able to inactivate ***intact*** HIV-1

retroviruses, while membrane-impermeant disulfide compounds, even with excellent NC cross-linking activity (*e.g.*, DTNB), had no effect.

9d. Similarly, the graph in Figure 15B[2] extends the findings of Figure 15A by showing that the highly effective ability of FDA, E4b, E1b, C3d, and C4d to penetrate the viral envelope and disrupt NC protein structure in ***intact*** HIV-1 retroviruses was associated with the ability of increasing concentrations of FDA (filled circle), E4b (filled square), E1b (open triangle), C3d (open circle), and C4d (open square) to inactivate ***intact*** HIV-1 retroviruses. By contrast, the reduced monomeric form of FDA ("Mon," filled square with dashed line) had no effect on inactivating ***intact*** HIV-1 retroviruses. As such, these results show that membrane-permeant disulfide compounds with excellent NC cross-linking activity (*e.g.*, FDA, E4b, E1b, C3d, C4d) were able to inactivate ***intact*** HIV-1 retroviruses, while reduced monomeric forms of these disulfide compounds with no NC cross-linking activity (*e.g.*, Mon) had no effect.

10. In view of the discussion above, I believe that the specification, *e.g.*, Example 4, describes a specific set of tests for readily determining whether a disulfide compound of interest is capable of inactivating an ***intact*** retrovirus by disrupting NC zinc fingers. I also believe that amended claim 24 by definition excludes any NC zinc finger-disrupting compound such as DTNB that has no effect on inactivating an ***intact*** retrovirus. Any such compound would simply fall outside the scope of the claim.

The declarant has nothing further to say.

Louis E. Henderson

Louis E. Henderson, Ph.D.

12/15/04
Date